XX.—*On the Germination of the Resting Spores, and on a form of Moving Spores in* Spirogyra. By Dr. W. Pringsheim*.

[With two Plates.]

While the observations on the conjugation of *Spirogyræ*, first made by O. F. Müller†, have since been frequently repeated and are now universally known, the germination of the spores produced through the conjugation, first seen by Vaucher‡ in 1803, has been confirmed only by very few subsequent observations. Considering the active interest which has recently prevailed in regard to the development of the Algæ, and has existed respecting the formation of the spores of *Spirogyra* itself, in a wider circle than that which merely includes algologists, the above fact is the more remarkable, since it is by no means difficult to procure the material required for the investigation; for although conjugation takes place most frequently in spring, I have found *Spirogyræ* both in a state of conjugation and preparing for it throughout the summer and until late in autumn. Nevertheless, so far as I know, there exist only three publications on the germination of the *Spirogyræ*, exclusive of course of all those which do not rest on original observations §.

The first, as already mentioned, was furnished by Vaucher, to whom we must always go back, when we are studying the development of the freshwater Algæ. He gave a representation of the germinating spores correct in all essential points, but not adequately good and accurate for the demands of our own day. These figures || are all botanical literature possesses. The essential part of his description of the germination is as follows: "The spores open at one end, like the cotyledons of a seed when its embryo is beginning to unfold, and the young plant emerges as a small, rapidly-growing green sac, in the interior of which the spiral bands, with thin shining granules (the starch-granules) and septa, soon present themselves. Finally the young plant leaves the envelope in which it originated, grows up in the water and then resembles the parent plant, excepting that its two ends are attenuated to points, and that it is of smaller size."

Meyen confirmed these observations. In one essay, indeed, which he wrote on the genus *Spirogyra* in 1827, he held Vaucher's observations on the germination of *Spirogyra* to be false.

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* From the 'Flora,' Aug. 14th and 21st, 1852: translated by Arthur Henfrey, F.R.S., F.L.S.

† Flora Danica, tab. 883.

‡ Histoire des Conferves d'Eau douce; Genève, 1803.

§ See Rev. W. Smith, 'On the Germination of the Spores of *Conjugatæ*,' Annals of Nat. Hist. ser. 2. viii. 480.—A.H.

|| *L. c.* tab. 4, 5, 6.

"Nevertheless"—he says there*—"it is more than probable that the observations made by Vaucher were not characterized by that great accuracy which is needful here, since, as the figures given show, the growth of these young Confervæ is in contradiction to all analogy, and it is therefore very necessary to repeat these investigations." But he must have been subsequently convinced by his own observations of the correctness of Vaucher's statements, for in his 'Manual of Physiology†,' he gave a description of the germination, which, while devoid indeed, as the limits of a manual compel, of that requisite detail which constitutes the value of a monograph, represents all the essential points of the phænomena so truly, that I have only to confirm all that he states of it in *this* place.

The third confirmation comes from Alex. Braun, in his recent work, 'Observations on the Phænomena of Rejuvenescence in Nature‡.' He mentions here, in several places§, not only the changes of the contents of the spores preceding germination, but the phænomena of the commencement of germination, the dehiscence and the stripping off of the spore coats.

Opposed in appearance to these exact observations on the germination of the bodies originating in the conjugated cells of *Spirogyra*, stands the statement of Agardh ||, that these bodies are broken up into moving spores after a certain time; on which account Hassall¶, who participates in this view, considers these bodies, not as spores, but, as the sporangia of the *Spirogyræ*. Unfortunately, the short account of Agardh, which, although the subject well deserved it, was not accompanied by drawings, does not allow of satisfactory conclusions as to the phænomena observed by him. Meyen** had already noticed that secondary —*but not moving*—cells were often formed inside the spores of *Spirogyra*, and he conjectured that there were likewise propagative cells. I have also found these secondary cells, in which

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* Linnæa, 1827, p. 421. † Pflanzen-Physiologie, iii. 422–424.

‡ Beobachtung üb. die Erscheinungen der Verjüngung in der Natur.

§ *Loc. cit.* pp. 144, 192, 215, 216.

|| The passage runs (Ann. des Sc. Nat. 2nd Ser. vi. 197): "After many vain attempts to see the elliptical body developed into a new filament, as described by Vaucher, *I saw it, on the contrary, broken up definitively into numerous sporules endowed with a rapid motion.*"

¶ History of British Freshwater Algæ, p. 130.

** "In fig. 13. pl. 10. are represented similar seeds (*Samen*) of *Spirogyra princeps*, which *have been formed without conjugation*, and this is very general in *Spirogyra quinina*; they also exhibit double coats; but the mass in their interior has been transformed into small vesicles, which probably may likewise be spores, the further behaviour of which, however, I have not seen. But the formation of these little vesicles in the true seed is not always to be met with in these *unconjugated* Confervas, and usually the green mass is spirally arranged here also."—*Meyen, loc. cit.*

the contents are frequently transformed into *spores not directly germinating*, in spores which had originated through copulation (Pl. VIII. fig. 7). They were always however motionless, and I was equally unsuccessful in observing a further development of these cells, and confirming the very natural conjecture of Meyen by direct observation. But I also frequently found the contents of the filament-cells—*when no large spore had been previously formed in them*—transformed into peculiar cells (Pls. VIII. and IX. figs. 4 & 8), which appear as the mother-cells of smaller *moving cells*; and the latter appear to stand in close relation to the development of the *Spirogyræ*. How far the phænomenon observed by Agardh agrees with one of these phænomena, will be seen from the subsequent description of my observations. At the same time, the import of the well-known large isolated bodies originating from the entire contents of one or two conjugated filament-cells (fig. 1 *a, b, c*), as true spores of the *Spirogyræ*, is not affected by the possibility of a propagation of the *Spirogyræ* by means of the secondary cells originating in the elliptical spores, since in the regular course of vegetation, the former, exactly as Vaucher observed, exclusively effect the propagation by their direct germination. The dissolution of the contents of a spore *capable of direct germination*, into daughter-cells equally *capable of germination*—for which Agardh's observation would speak—as well as the occurrence generally of *several different* forms of spore in the same plant, appear to me only a result of the independence of the individual cell prevailing in the Algæ, and a very general property of these, physiologically speaking, simply unicellular plants. I shall return to this point in speaking of the rare forms of spore of the *Spirogyræ* at the conclusion of this memoir.

I observed the germination of the ordinary form of *Spirogyra*-spores, those well-known large, elliptical bodies, in *Spirogyra jugalis**. Conjugated specimens of this *Spirogyra*, collected in August, maintained themselves in this condition through the winter, in my room, in a little glass vessel full of water, to the bottom of which they gradually sank. Some spores germinated as early as February, but most of them did not open until April, so that some eight months elapsed between their formation and their germination. We observe in the spores of *Spirogyra*, as in all motionless spores of Algæ, a long period of rest between for-

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* The determination of the name was made with Kützing's 'Species Algarum.'—The plant I examined had several, mostly 4, spiral bands; the septa of its cells were not thrown back in folds (see, in regard to such folds in *Spirogyræ*, Cohn's Essay in Nova Acta Acad. N. C. xxii. pars ii. 250 *et seq.*). The diameter of the filaments was 0·1 millimetre; the length of the joints, fertile and barren, varied between 0·12 and 0·2 mm.; some attained a size of 0·3–0·4 mm.

mation and unfolding*; yet during this time of *apparent* rest, processes are unceasingly active in the interior of that germ, not immediately manifesting themselves to the eye, but resulting in effects, which may be detected in the spores of *Spirogyra* in demonstrable alterations of the contents and of the membranes of old spores. Immediately after formation, the spore possesses only one single, perfectly colourless, thin membrane, which, as is shown by the acquisition of a blue colour with iodine and sulphuric acid†, is composed of pure cellulose. In many spores, this membrane is still so thin for a short time after the formation of the spore, that it is yet incapable of withstanding the strong endosmose excited by the addition of sulphuric acid, and bursts at some point, allowing the escape of the contents. The contents of the new-formed spores consist of the almost unchanged spiral bands of the cells concerned in the formation of the spore. The spiral bands are, indeed, contracted far more closely together than in the filament-cells, but retain even their form scarcely changed. As in the spiral bands of the *Spirogyra*-cell, so also the spores contain numerous large and small starch-granules, lying in a layer of the so-called amorphous chlorophyll (extractable by absolute alcohol), which appears to be deposited upon the finely granular protoplasm (a mixture of oil and proteine-substance, albumen ?), visible after the removal of the chlorophyll. The older the spores grow, the more does the form of the spiral bands in their interior disappear, and their contents become uniformly diffused over the entire inner surface of the spore-membrane. Finally, just before the germination, the original spiral arrangement of the contents is still indistinctly indicated by several close spiral streaks in the coating, spread uniformly over the wall (fig. 1 *a*, *b*, Pl. VIII.). It is a peculiar circumstance, that during this time the spiral arrangement of the contents of the spore presents itself, sometimes distinctly and sometimes indistinctly, and almost wholly vanishes at the moment of germination, but always appears with surprising clearness when the spores are left for some time in glycerine, or are allowed to become perfectly dried up (Pl. VIII.

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* This long repose between formation and development is perhaps the only character which the spores of the Cryptogamia have in common with the seeds of the Phanerogamia. But the true analogue also of the Cryptogamic spore in the Phanerogamia, the pollen, is well known to be capable of maintaining its germinative form through long periods of rest.

† I prefer the application of iodine and sulphuric acid to the apparently more convenient use of the so-called chloride of zinc solution (chloride of zinc, iodine and iodide of potassium), since the former is a much *stronger* and more certain reagent for cellulose, and produces the blue colour without previous application of an acid or an alkali, even in cases where the chloride of zinc solution is ineffective.

fig. 5). Chemically speaking, the contents of the spore appear to be more changed in the relative proportions of quantity of the particular constituents, than in their quality, before germination. The principal constituent consists of large drops of oil, becoming confluent under pressure, with amorphous chlorophyll and albumen, as in the newly formed spore. The *large* starch-granules have disappeared and are replaced by very small irregular corpuscles devoid of any distinguishable structure, but which become blue when iodine is applied, and, therefore, are likewise starch. Lastly, as an entirely new constituent of the spore, appear certain reddish-brown corpuscles, *never absent*, which are also found in the young plant after the germination (Pl. VIII. fig. 1 *d*, fig. 5, fig. 2 *d*).

The differences between the membranes of old and young spores are more important than the changes perceptible in the contents.

Instead of the *one* colourless cellulose membrane of the young spore, this latter exhibits, shortly before germination, three distinct membranes, not blended together. The inmost encloses the entire contents, which are already surrounded by the primordial utricle; the outermost (*e*, fig. 1 *a*, *b*, and fig. 5), thin and colourless, is composed of pure cellulose, as may readily be demonstrated by iodine and sulphuric acid; it is the same membrane which the spore possessed at the time of its formation, only it has become thicker, without, however, perceptible lamellation. Within this lies, without touching it at all points, but closely applied to it, the second coat, a membrane of yellowish-brown colour (*f*, fig. 1 *a*, *b*, and fig. 5), which retains its colouring matter with great obstinacy, and *is not coloured blue by sulphuric acid and iodine.* This, finally, encloses the third, inmost and last-formed membrane, which is colourless like the first, and is also coloured blue with iodine and sulphuric acid. This third membrane is not always visible in the unopened spore, and hence, perhaps, has remained unnoticed by previous observers; perhaps, however, from its only appearing shortly before the germination, as the last deposit of membrane within the spore. It constitutes, really, with the contents it encloses, the essential part of the spore-cell, since in the germination of the spore it grows out directly into the young plant, after the dehiscence and casting off of both the outer membranes. Its existence may always be ascertained by bursting oldish spores by slight pressure, and allowing their contents to escape gradually. If the spore thus burst by pressure is afterwards treated with iodine and sulphuric acid, the third inmost membrane assumes a blue colour, and in this way only can it be certainly made out that the blue colour belongs really to it, and does not depend on the outer coat of the spore or the membranes of the cell in which the spore perhaps still lies. The detection of the three membranes of the spore is very readily effected by the application of concentrated potash. The spore does not burst when left in concentrated solution of potash, but after a few days, the three membranes appear clearly separated from each other (fig. 6 *a* & *b*). Under these circumstances, the inner cellulose membrane (*g*, fig. 6 *a* & *b*) exhibits the remarkable property, otherwise found only in the primordial utricle, of contracting by shrivelling together. It surrounds the primordial utricle (*h*, fig. 6 *b*) with its contents contracted into the middle of the cavity of the cell. The shrivelling together of this cellulose membrane is often so strong, that it is no longer capable of holding the contents, and these, dissolved by the potash, escape in large drops, of indefinite form, into the interspace between the inmost and the middle yellow membrane (*i*, fig. 6 *b*). And in the spores treated with potash, after the latter has been washed out, the application of iodine and sulphuric acid turns this third inmost membrane (*g*, fig. 6 *a* & *b*) bright blue, so that there can be no doubt of its chemical constitution.

The *production* of the two inner membranes in the spores takes place in exact analogy to the universal formation of secondary layers of thickening in vegetable cells; the middle, yellow coat follows the outer primary coat, not only in position but in structure, as a secondary deposit, and the deposition of the inmost, in regard to its productive *tertiary* cellulose membrane, occurs long after the formation of the yellow coat. Since Mohl's* researches have demonstrated that cellulose is the basis of the thickening layers of all vegetable cell-membranes, its reaction, frequently hidden by infiltrated matters, reappearing clearly after the removal of them by potash or nitric acid; it was natural to conjecture that the yellow, middle membrane of the spore would exhibit the cellulose reaction if properly treated. But I only succeeded in demonstrating the cellulose in this membrane after much trouble, for all the means I applied to extract or destroy the colouring matter of this membrane were at first ineffectual. Only after a longish digestion in *aqua regia* was the yellow spore-membrane bleached, without being destroyed. When the *bleached* spores, well washed with water to remove the *aqua regia*, were treated with *iodine* and *sulphuric acid*, the thick, middle, previously yellow membrane became blue. The more perfectly the membrane was bleached by *aqua regia*, the purer the blue colour acquired with iodine and sulphuric acid; the less perfect the bleaching was, the more the blue inclined to green. This membrane certainly is one of those vegetable membranes in which it is most difficult to demonstrate the well-

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* Botanische Zeitung, 1847; Trans. in Scientific Memoirs, vol. i. 2nd ser.

known cellulose reaction,and therefore offers a strong support to the opinion that the cellulose reaction is only prevented in membranes which do not exhibit it, by a matter infiltrated in them. In one case the infiltrated matter may be detected even by its colour, and after the removal of this substance the membrane reacquires, with the ordinary colourlessness of vegetable membrane, the chemical characters of cellulose.

After the transformation of the contents is terminated and the formation of the inner two membranes completed, the germination of the spore commences by a growth of the internal cell formed by the inmost membrane. The increasing size of the internal cell first causes the yellow membrane to break across in an irregular crack (Pl. VIII. fig. 1 *a*), and after a further growth of the germinating cell, the outer colourless membrane tears in a similar manner. *This* succession of the bursting of the outer coats of the spore is caused by the structure of the spore and the unyielding rigidity of the middle coloured coat. The internal cell, bursting forth from the coats, grows in the course of a few days into a longish cell, which soon presents septa and becomes a many-celled filament, which resembles the parent, both in the number of spiral bands and in dimensions (Pl. VIII. fig. 1 *c*)*. Even in the unicellular condition, one end of the cell is elongated in a tubular form (fig. 3). The green spiral bands do not extend into this, always unbranched, radical extremity, and its further growth being restricted, it remains fixed from an early epoch, at *that* stage of development which it has attained in the young, few-celled plant, while the opposite end of the spore is capable of unlimited elongation by uninterrupted growth and repeated formation of septa.

This *differencing* of the two ends of the spore, expressed in different directions of growth, and the limited growth of one and the unlimited growth of the other, occur indeed—with the *very rare* exceptions when both ends are characterized by unlimited growth—in all spores; but a difference is found in them, that while, in most, that end of the spore-cell which emerges first out of the coats (figs. 1, 2, 3, 10), is converted into the cellular *Spirogyra*-filament, and the end remaining in the coats grows out into the radical tube, in other (less numerous) spores, their two ends behave in exactly the opposite way, the cell-forming end remaining behind in the coats (Pl. IX. fig. 11 *a, b, c*), and the radical extremity making its way out of

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* Hence the characters derived from the number of the spiral bands, and the dimensions of the filament-cells appear to have a specific value; at all events these characters are propagated by germination. Compare also Vaucher's figures of the germinating *Spirogyræ* with those of the parent plants.

them. In spite of this difference, however, the young plants produced exhibit exactly the same behaviour in both cases.

I had conjectured at first that the opposition between the anterior and posterior extremities of the spore would be already indicated by its position in the filament-cell. All spores of the same filament open ordinarily on the same side, so that if we call *that* end of the spore through which the young plant emerges, the anterior extremity, all the spores of one filament have their anterior ends turned in the same direction (Pl. VIII. fig. 1 *a*, *b*, *c*). But I afterwards remarked that no constancy prevailed in this, for I met with filaments, rarely it is true, in the cells of which the anterior ends of spores were turned to opposite sides (Pl. IX. fig. 9), so that it could not be certainly determined in the unopened spores, which was the front and which the back. It need scarcely be mentioned, that accidental twisting of a filament was taken into account here. The end of the young plant, no matter whether it was the radical extremity or the growing summit, remained sticking in the burst coats (fig. 1 *c*, 11 *c*) long after the emergence of the other end, and the envelopes were not thrown completely off until a late period, and then either accidentally, or, as mostly occurred, by the young plant rising from the bottom of the water, where the germination took place. I never saw the liberated young plant become attached to anything by its radical extremity, and this corresponds to the ordinary floating condition of the *Spirogyræ*. But I cannot decide whether or not the *Spirogyræ* become fixed to anything by their root-cell, at a later stage than that to which I was able to trace the young plants*. It is probable, however, that those *Spirogyræ* which are found adherent in their natural stations, use their root-cell as the organ of attachment. At the same time, the somewhat elongated basal cell, enlarged below into a shield-shaped root, described by Nägeli as occurring in the Zygnemaceæ†, is certainly not the root-cell produced in the germination, but one of the ordinary filament-cells, enlarged into a short colourless expansion at one extremity. Whether the filament-cell thus altered is incapable of propagation, as Nägeli asserts, I am inclined to doubt, since it is in any case certain that the true root-cell produced in germination is capable of transforming its contents into propagative cells in the shape of moving spores.

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* One of the largest of the young plants which I obtained in a perfectly healthy condition was 2·6 mm. long. It was composed of thirteen cells of tolerably equal length, excepting that the root-cell was longer; so that the length of these (subsequently dividing) cells of the young plant equalled that of the larger, undivided (?) cells of older plants.

† Gattungen einzellige Algen, p. 4.

The germination of the spores also gives some insight into the origin of the spiral bands.

When the spore breaks out, the contents form a coat uniformly spread over the wall, with only a slight indication of spiral arrangement (*a, b,* fig. 1. Pl. VIII.). As the young cell grows, this becomes broken up, and the originally irregular and imperfect slits thus produced, subsequently cut in a continuous course through the originally uniform coat, which is now slit up into regularly arranged bands (Pl. VIII. figs. 2, 3). The cause why the coating of the wall tears up into spiral and not rectilinear bands, remains unknown here, just as in the origin of all other spiral forms in the vegetable cell. Germinating plants of *Spirogyræ* with only one spiral band, might, perhaps, give an opportunity of discovering more accurate particulars of this process. That the cytoblast—Meyen's 'central-organ'—notwithstanding the mucilaginous filaments running out from it to the borders of the spiral bands—plays no part here, seems to me so much the more probable, that I doubt its actual existence in the spore and in the young unicellular plant. I never found the cytoblasts in the spores, *even when the contents were gently pressed out, which would make it clearly visible,* and just as little could I detect it in the much more transparent young, unicellular plant (figs. 2, 3). It is first found in the *two-celled* plants, and many-celled specimens have one in *each* cell, even the *radical cell;* it is not oval, but round (fig. 1 *c, m, m, m*). Alex. Braun has shown the part it plays in the formation of new cells in the *Spirogyra**. It appears, therefore, *that it originates* in the unicellular plant *immediately before the formation of the septum,* and then quickly causes the formation of two new cytoblasts, either through solution or subdivision, and thus we should bring its presence in *all* cells of old plants into agreement with its absence from the spores and unicellular plants.

[To be continued.]

XXI.—*Revision of the Families of Nudibranch Mollusks, with the description of a new Genus of* Phyllidiadæ. By J. E. Gray, Ph.D., F.R.S., V.P.Z.S. &c.

The very important results which were obtained by the examination of the tongues and teeth of the *Ctenobranchous* Mollusca, which were partly published in the last Number, have induced me to continue my researches on these organs in the *Nudibranchiate* Mollusca. They have resulted in two important facts:

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* *Loc. cit.* p. 257 *et seq.*